EXHIBIT H

Histologic Comparison of Pubovaginal Sling Graft Materials: A Comparative Study

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OBJECTIVES Little is known about the host response to the various biologic and synthetic graft materials used as substitutes for autologous fascia. We investigated the host response to sling graft materials in humans.

METHODS A total of 24 women undergoing sling revision had a portion of the graft material removed for comparative analysis. At exploration, the degree of graft preservation (integrity), encapsulation, infection, and fibrosis was quantified. A histopathologic analysis was performed by systematically examining each specimen for the inflammatory response, neovascularity, and host fibroblast infiltration.

RESULTS A total of 24 grafts were explanted at 2-34 months after implantation. The indications for removal were a lack of sling efficacy in 2, urinary retention in 9, and sling obstruction in 13. The types of graft material were polypropylene mesh (PPM) in 10, autologous fascia in 5, porcine dermis in 4, cadaveric dermis in 3, and cadaveric fascia in 2. No graft degradation had occurred in PPM material. Autologous and cadaveric fascia had the most demonstrable graft degradation. No encapsulation had occurred with autologous fascia or PPM. The porcine dermis was the most encapsulated. No host infiltration had occurred with the encapsulated porcine grafts, and only peripheral infiltration of fibroblasts had occurred in the cadaveric grafts. The PPM grafts had the greatest number of fibroblasts throughout the entire graft. Neovascularity was the most prevalent in mesh and was also present in the autologous fascia. Giant cells were seen in two mesh and two porcine grafts.

CONCLUSIONS The results of our study have shown that porcine dermis has the potential to encapsulate. The degree of host tissue infiltration was greatest with PPM, and no degradation of the mesh material had occurred with time. UROLOGY 72: 85–89, 2008. © 2008 Elsevier Inc.

Stress urinary incontinence is a very bothersome condition that affects 10%-20% of the female population.[1] The surgical treatment of stress urinary incontinence has evolved during the past several decades from retropubic and transvaginal urethral suspension procedures to the primary use of sling procedures. The American Urologic Association Stress Urinary Guidelines Panel determined that pubovaginal slings and retropubic suspensions were most efficacious in the treatment of stress urinary incontinence.[2] Chakin et al.[3] demonstrated the successful use of a pubovaginal sling in women presenting with all types of stress urinary incontinence. Subsequently, pubovaginal sling procedures became accepted as the reference standard in the surgical management of stress urinary incontinence, and several investigators have reported the long-term efficacy and safety of the procedure.[4–6] To minimize the morbidity of graft harvest, biologic and synthetic graft materials have been increasingly used in sling surgery. Decreased perioperative pain and hospital stay have been associated with the use of graft substitutes.[7] Despite the encouraging early results, some data have suggested greater intermediate and late failure after biologic sling procedures.[8,9] Synthetic slings, although associated with excellent early results, have been reported to be sources of infection and occasional urethral erosion.[10]

With the emerging use of graft substitution materials, an increased knowledge of the host response to these materials is needed. Insufficient data are available to assess the host response to these materials after implantation. These data can have a variety of implications regarding efficacy and safety. Therefore, we sought to compare the histopathologic characteristics of these various sling materials after explantation during sling revision surgery. Perhaps by comparing the changes in the host–graft relationships of these various materials, we might be better able to understand the

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potential clinical significance of using biologic and synthetic sling grafts.

MATERIAL AND METHODS

A total of 24 consecutive women undergoing sling revision surgery had portions of their slings removed at explantation for the following indications: lack of efficacy in 2, urinary retention in 9, and sling obstruction in 13. The patients were classified as obstructed if they had persistent lower urinary tract symptoms and had a clinical diagnosis of obstruction as a result of the sling procedure. Patients in retention were those reliant on intermittent catheterization, which became necessary after the sling procedure. These graft explantations occurred at two sites: Vanderbilt Medical Center and the Ochsner Clinic Foundation. During exploration, each graft was examined and graded systematically by the explanting surgeon. Each graft was inspected for signs of encapsulation, infection, fibrosis, and degree of preservation (integrity). Encapsulation was defined as a fibrous rim of tissue surrounding and isolating the graft material. Encapsulation was quantified from no encapsulation, which consisted of the graft within the host tissues, to severe encapsulation, consisting of a thick capsule completely isolating the graft material. Infection was defined as gross evidence of purulence or cellulitis consistent with a clinical infection. Degradation was defined as a loss of graft structure, ranging from no degradation, which is characterized by no loss of graft thickness or structure to severe degradation, in which thinning of the graft and breakdown of the graft structure had occurred, disrupting the scaffold of support.

After gross examination, these samples were placed in formalin solution and underwent conventional hematoxylin-eosin staining procedures. Similar sectioning techniques were used for the various material types. Each specimen was then systematically examined microscopically by a pathologist (E.N.B.) who was unaware of the material type. The pathologist specifically inspected each graft to quantify neovascularity, inflammatory response, host fibroblast infiltration, and areas of necrosis. Neovascularity was defined as the presence of blood vessels within the graft. Blood vessels were defined as endothelial-lined vessels containing erythrocytes. Inflammation was identified by the quantification of white blood cells, macrophages, or foreign body reaction (eg, giant cells). Host cellular infiltration was identified by the quantification of fibroblasts within the graft material.

The patient records were reviewed for any host factors that could potentially inhibit graft remodeling. These factors included age older than 70 years, diabetes, steroid use, smoking history, and a history of graft infection/complications. All variables were analyzed systematically by comparing each graft material. Additional analyses of the gross and histopathologic characteristics of the graft materials were compared according to the interval from surgery at which the material was extracted.

RESULTS

A total of 24 grafts were explanted 2-65 months after implantation. The types of materials explanted included polypropylene mesh (PPM) in 10, cadaveric fascia in 2, cadaveric dermis in 3, porcine dermis in 4, and autologous fascia in 5. The average age of the patients who underwent explanation was 60.3 years for those with PPM, 58.6 years for those with cadaveric dermis, 62.8 years for those autologous fascia, and 66 years for those with porcine dermis. A trend was noted for advanced age in the porcine dermis group, reflective of selection bias. No patient had been taking steroids chronically, and none had had a history of graft infection or rejection before the study. Tobacco use was present in 30% of PPM, 0% of autologous fascia, 20% of cadaveric dermis, and 50% of porcine dermis patients. The porcine dermis patients had a greater frequency of tobacco use.

On gross inspection, the autologous fascia grafts demonstrated only moderate degradation; however, the integrity of the grafts appeared intact, with no compromise of the graft scaffolding. The autologous material displayed no evidence of encapsulation or gross infection. Microscopically, the autologous fascia showed moderate and uniform infiltration of host fibroblasts, as well as neovascularization. No evidence of foreign body reaction was evident, with no inflammatory cell infiltrate.

The porcine dermis grafts were grossly free of degradation or thinning and displayed an appearance very similar to that at implantation. Each was severely encapsulated and completely separate from the periurethral tissue. As might be expected from their gross appearance, these grafts microscopically appeared completely acellular without any evidence or neovascularization or host infiltration.

The cadaveric tissues demonstrated the most degradation of all harvested materials, as well as mild to moderate encapsulation. The microscopic specimens demonstrated host infiltration of fibroblasts only at the periphery of the grafts, with the central portion of all but one specimen remaining acellular. All grafts were free of neovascularization.

The PPM explants displayed no evidence of degradation or encapsulation and had the greatest degree of host tissue infiltration. Microscopically, host infiltration was abundant and displayed throughout each graft. These grafts demonstrated the greatest degree of neovascularity. A foreign body reaction was also evident by the presence of giant cells, macrophages, and occasional calcification. A summary of the comparison of graft materials is included in Table 1 and Figures 1 and 2.

When the grafts were analyzed according to the interval after surgery, similar changes were noted. Over time, the degradation appeared progressive in the patients with cadaveric grafts. This appearance was fairly consistent throughout all intervals, with the exception of one cadaveric fascia graft that had the presence of fibroblast infiltration throughout the entire graft 38 months after it had been implanted. Despite this, we were able to localize all sling grafts in this group of patients. Other graft materials did not demonstrate this trend of progressive degradation with time.

COMMENT

As pubovaginal slings gained widespread acceptance in the surgical management of stress urinary incontinence, the use of grafts as a substitute for autologous fascia has

Table. Gross and histopathologic comparison of graft materials

Graft material	Patients (n)	Graft Degradation	Encapsulation	Infection	Host Fibroblasts (Location)	Neovascularity
PPM	10	None	None	None	Many (uniform)	Moderate
Cadaveric fascia	2	Moderate	None	None	Few (peripheral)	None
Cadaveric dermis	3	Mild	Mild	None	Few (peripheral)	None
Porcine dermis	4	None	Severe	None	None	None
Autologous fascia	5	Moderate	None	None	Moderate (peripheral)	Few

PPM = polypropylene mesh.

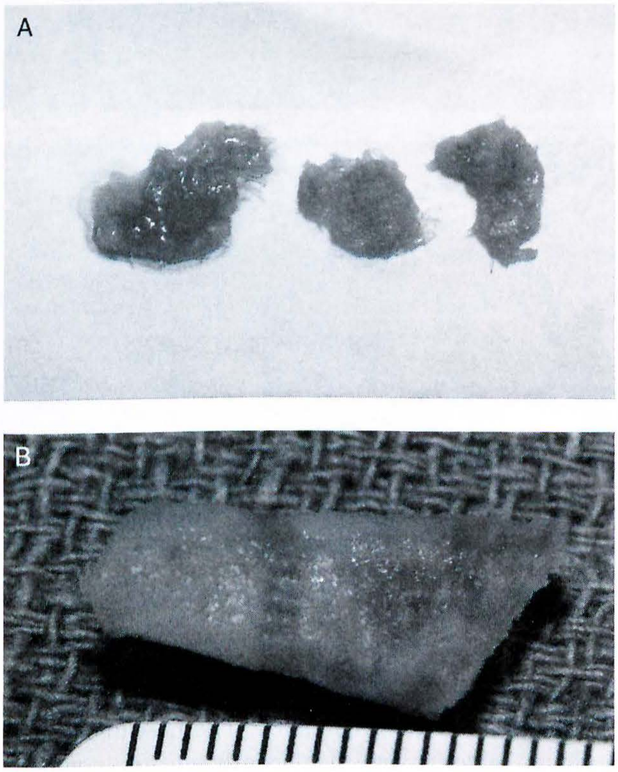

Figure 1. Variance of gross appearance of graft material at explanation: (**A**) significant infiltration of host tissue in polypropylene mesh, (**B**) lack of host infiltration in porcine dermis.

become commonplace. First introduced by Jarvis and Fowlie,[11] using porcine dermis, these investigators reported cases of "vaginal weeping." The practice became widely accepted after Handa et al.[12] described using cadaveric fascia lata, which was readily available from many tissue banks. Initially, the results using these materials were encouraging. However, several subsequent reports[9,10,13] of intermediate and late sling failures with these materials led to concerns regarding the use of biologic grafts as sling substitutes. The use of PPM offers an attractive advantage, but concerns regarding infection, foreign body reaction, and erosion exist. Few data are available regarding the biocompatibility of these materials—particularly after transvaginal implantation. The industry standard of biocompatibility testing requires subcutaneous placement of materials. Does this translate to biocompatibility after transvaginal implantation? As we continue to debate the ideal graft to substitute for autologous fascia, we must have a better understanding of their biocompatibility and acceptance by the host with time.

To better understand the graft–host relationship, we systematically examined the histopathologic characteristics of various graft materials after they were explanted from human subjects undergoing sling revision surgery. Using similar tissue processing and staining techniques, these samples were examined systematically and compared with each other. Our examination revealed significant differences in the gross and microscopic findings in the various materials. Autologous fascia had the greatest degree of host fibroblast infiltration with minimal inflammatory or foreign body reaction. This material was consistently intact, with a small amount of sling degradation at explantation. In contrast, the cadaveric dermis and fascia had little host fibroblast infiltration and little neovascularity, particularly within the central aspects of the graft. In addition, inconsistencies were found with this material grossly, with most specimens exhibiting significant thinning and degradation of the graft, disrupting the sling scaffold. Synthetic materials actually demonstrated the greatest amount of fibroblast ingrowth and tissue ingrowth into the specimen. Grossly, the mesh lattice was completely incorporated with viable host tissue. No degradation or disruption of the graft was found, and the substance of the graft was completely infiltrated by host tissue. Microscopically, the synthetic material had large amounts of fibroblasts and also exhibited a foreign body reaction characterized by giant cells and occasional calcification. Although the foreign body reaction was visible microscopically, no gross evidence was found of graft disruption or adverse effects on the host because of this foreign body reaction. Finally, the porcine dermis materials had the greatest propensity to encapsulate. The porcine dermis had a rind around it, which isolated the graft from the periurethral tissue. In addition to this, no host fibroblast infiltration, no inflammatory reaction, and no foreign body reaction was found. This was presumably because this material was walled off, with no access of the host to the material. The substrate of the graft was intact; in fact, the graft appeared similar to its original appearance at implantation.

Although this study did not correlate clinical outcomes, perhaps the histologic findings reflect some of the present controversy. The intermediate failures of slings using cadaveric materials have been previously described,[14] with the material being thinned or absent. As

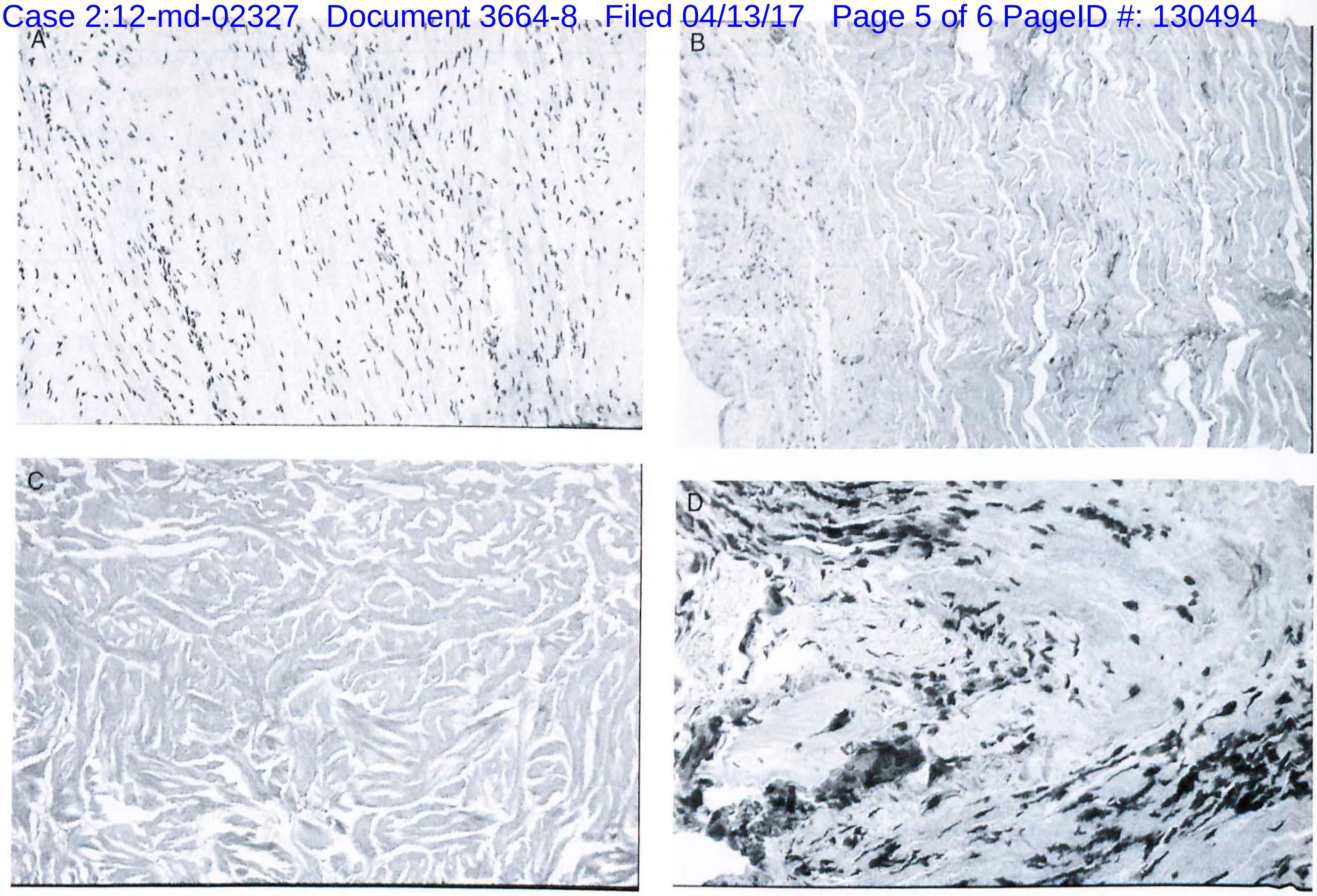

Figure 2. Variance of microscopic appearance of graft materials: (**A**) autologous fascia, (**B**) cadaveric fascia, (**C**) porcine dermis, (**D**) polypropylene mesh.

in our study, the degradation of these materials has been reported. The porcine dermis grafts also had a propensity to encapsulate, as previously reported in various studies.[15] This could ultimately affect the long-term viability of this graft material and also create potential complications such as pain and/or urinary retention.[16] However, the implications of encapsulation are largely unproved. Our data suggest that the tissue ingrowth in synthetic material is significant. Degradation of this material was not seen, particularly compared with that present in the cadaveric materials in this study. This implies that the synthetic materials are durable. To date, no significant data have demonstrated intermediate failures with these procedures.

The limitations of this study were significant. First, our study lacked standardization of the histopathologic findings regarding host remodeling. No uniform grading system is available that can be used to compare these various materials. This is clearly needed to facilitate an accurate comparison of studies of the histologic features of these materials. Second, the graft materials were not explanted at definitive points after implantation. Such a study is unlikely to be performed in human models because this would require removing grafts in women without symptoms. However, this could have affected the variance in the remodeling of our specimens.

Despite these limitations, we believe these data have clearly demonstrated that the human body reacts to these various sling materials differently. The host ingrowth in synthetic material was significantly greater compared with that with biologic materials. The clinical implications are unknown, but our results clearly indicate that additional investigation into host tissue remodeling is warranted. An animal model that replicates transvaginal insertion is needed to facilitate controlled comparisons. Additionally, consensus is needed on how to examine these materials after they are explanted from human subjects to gain a better understanding of the host response to these tissues.

CONCLUSIONS

The results of our study have demonstrated that porcine dermis has a propensity to encapsulate, which we assert could retard host infiltration into the graft. The degree of host infiltration was greatest in PPM. Considerable research is needed to understand the human host response to the various graft materials used for pubovaginal sling surgery.

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